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Impact of CRISPR immunity on the emergence and virulence of bacterial pathogens

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Abstract

CRISPR-Cas systems protect prokaryotes from viruses and plasmids and function primarily as an adaptive immune system in these organisms. Recent discoveries, however, revealed unexpected roles for CRISPR loci as barriers to horizontal gene transfer and as modulators of gene expression. We review how both of these functions of CRISPR-Cas systems can affect the emergence and virulence of human bacterial pathogens.

Introduction

Outnumbered by their viral predators by a factor of 10 [1], prokaryotes have evolved a diverse assortment of defense mechanisms to survive bacterial virus (phage) attack. Historically, the best studied of such mechanisms include restriction modification, abortive infection, and toxin-antitoxin systems [2]. More recently, a prokaryotic immune system has been identified that uses small guide RNAs to combat phage and prevent the stable incorporation of mobile genetic elements [3–5]. This immune system is composed of clustered regularly interspaced short palindromic repeat (CRISPR) loci and flanking CRISPR-associated (*cas*) genes (Fig. 1).

While CRISPR-Cas immune systems in different organisms exhibit marked phylogenetic and mechanistic diversity, they all block the stable entry of foreign nucleic acids in three common steps: adaptation, CRISPR RNA (crRNA) biogenesis, and targeting (Fig. 1). During adaptation, viral or plasmid challenge stimulates the incorporation of short (24–48 nucleotide) invader-derived sequences in between equally-short DNA repeats found in the CRISPR locus [6–9]. These unique sequences, called spacers, provide a historical account of past invaders and specify future targets of CRISPR immunity. Although the majority of spacers match viruses and other mobile genetic elements, many also match chromosomal sequences [10,11]. Therefore CRISPR-Cas systems seem incapable of discriminating between DNA molecules of different origins and have been proposed to act as a barrier to horizontal gene transfer [12]. During crRNA biogenesis, transcription of the CRISPR locus generates a long RNA precursor containing repeats and spacers in one contiguous array. This precursor is cleaved within repeats to separate individual spacers and liberate mature crRNAs that each define a single nucleic acid target [13–17]. During targeting, crRNAs

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assemble with Cas proteins to form a surveillance complex, which destroys invading genetic elements antisense to the crRNA it carries [18–20].

Considerable variations of the CRISPR immunity pathway and the Cas proteins that execute it have been observed in different organisms, thus spurring efforts to unite these systems under one simple classification scheme. Recent comparative analyses of Cas protein sequences, *cas* gene content, and genomic organization of CRISPR-Cas loci have lead to their consolidation into three distinct types (I-III) and eleven further subtypes (IA-F, IIA-C and IIIA-B) [21–23]. The only genes universally present in all CRISPR-Cas systems are *cas1* and *cas2*, which together mediate adaptation [9]. CrRNA biogenesis and targeting require various other *cas* genes, and specific signature genes have been identified among these to distinguish each type and subtype [24].

Figure 2 depicts the families of Cas proteins that mediate crRNA biogenesis and targeting in each of the three main Types of CRISPR-Cas systems. Types I and III systems exhibit the most similarities (Fig. 2 A and C). In both systems, crRNA biogenesis requires Cas6 family members to cleave the repeat sequences of the crRNA precursor, and targeting is facilitated by a large, multi-subunit ribonucleoprotein complex. The targeting complex in Type I systems is called CASCADE (CRISPR-associated complex for antiviral defense) [13], which contains a signature subunit from the Cas8 family, along with members of Cas5, Cas6, and Cas7 families. A distinct small subunit (SS, Fig. 2A), may also be present in Type I complexes. Nucleolytic cleavage of the target DNA is carried out by a Cas3 family nuclease, which is not a member of the complex. In Type III CRISPR-Cas systems, following the cleavage of the crRNA precursor by Cas6, crRNAs are further trimmed at the 3′ end by an unknown nuclease [16,25]. The main difference between Types III-A and III-B is the chemistry of the target nucleic acid: while genetic data indicates that Type III-A CRISPR-Cas systems cleave DNA molecules *in vivo* [26], type III-B CRISPR-Cas systems cleave RNA targets *in vitro* [19]. In both cases, targeting requires a large ribonucleoprotein complex, the Cas10-Csm complex in Type III-A [27] and the CMR complex in Type III-B [19]. Both targeting complexes possess the signature subunit Cas10, along with Cas5, multiple distinct Cas7 family members, and a distinct small subunit (Fig. 2C). The nucleases that attack DNA and RNA targets are yet unknown. In contrast to Types I and III systems, Type II systems require minimal Cas machinery for immunity (Fig. 2B). For crRNA biogenesis, these systems utilize a trans-encoded CRISPR RNA (tracrRNA), a small RNA that shares partial complementarity with CRISPR repeats [15]. Pairing between the tracrRNA and the precursor crRNA generates a double-stranded substrate that is cleaved by the host-encoded RNase III to liberate the small crRNAs. Cleavage of the DNA target in Type II systems is carried out by a single large multidomain protein, Cas9. This is an RNA-guided double-stranded DNase with two independent nuclease domains, HNH and RuvC, each of which cleaves one strand of the target DNA [28].

CRISPRs reside in bacteria and bacterial pathogens alike

Pervasive in the prokaryotic world, CRISPR-Cas systems have been identified in nearly all archaea and over 40% of bacteria, including numerous bacterial pathogens. This overall prevalence of CRISPRs in all sequenced bacteria is reflected in a subset of common bacterial pathogens: of 438 selected pathogenic strains reported in the CRISPRs database [29], a web-based tool that scores the CRISPR content in sequenced bacteria, ~45% (198) harbor at least one CRISPR system (Table 1). Type I CRISPR-Cas systems are the most common in these pathogens (37%, Table 1), congruent with the general abundance of Type I systems among all sequenced bacteria (~38%) [23]. Similarly, Type III systems are present in ~10% of the listed pathogens (compared to the 15% prevalence of Type III systems in all sequenced bacteria). In contrast, Type II systems are the least abundant in bacteria (found in

~10% of sequenced strains) [23], but seem to be twice as prevalent in our selection of human pathogens, residing in ~20% of them (Table 1). In the following sections, we review the studies that have addressed the role of CRISPR-Cas systems in human pathogens.

Staphylococci

Staphylococci are commensal opportunistic pathogens that reside on the skin and mucous membranes of humans. In recent years, pathogenic staphylococci that are resistant to all known antibiotics have emerged in both hospital and community settings [30] and have become a major threat to public health. The primary means of antibiotic resistance dissemination in staphylococci is through the horizontal transfer of conjugative plasmids [31]. *Staphylococcus epidermidis* RP62a, a clinical isolate, was found to harbor a Type III CRISPR-Cas system with a spacer that matches all sequenced staphylococcal conjugative plasmids [32]. This observation underscores the potential for this system to prevent the spread of such plasmids and the antibiotic resistance genes they carry. Experimentally, CRISPR immunity in *S. epidermidis* was shown to limit the uptake of a conjugative plasmid harboring a mupirocin resistance cassette [26]. Considering the enormous selective pressure applied by antibiotics upon staphylococci, this finding suggested that anti-plasmid CRISPR immunity could be detrimental for the evolution of these bacteria. In other words, CRISPR immunity could prevent the dissemination of antibiotic resistance mediated by conjugative plasmids that is so common between staphylococci. Alternatively, CRISPR-Cas systems could have a mechanism to distinguish “bad” DNA (such as an invading phage) form the “good”, antibiotic resistance-carrying, plasmid DNA. To address this possibility, a recent study analyzed the few CRISPR-escape cells that are able to acquire the plasmids that confer mupirocin resistance in spite of the presence of a targeting CRISPR-Cas system in the population of recipient cells [33]. All of the “escapers” analyzed (111) contained mutations in the CRISPR-Cas locus: 50 % lost the compete CRISPR-Cas region, 21 % contained transposon insertions in the *cas* genes, 16 % harbored single-nucleotide deletions or substitutions that abrogated Cas function, and 13 % of the cells lost the spacer sequence that matches the conjugative plasmid. These results indicate that the CRISPR system and its target cannot coexist in the cell; the only bacteria that survive the antibiotic selection have lost a functional CRISPR-Cas system. Therefore it can be speculated that the absence of CRISPR loci in most *S. aureus* strains (Table 1) is a consequence of the importance of plasmids, prophages and other mobile genetic elements for the virulence of this pathogen [34,35] and the ability of CRISPR-Cas systems to attack these elements.

Pneumococci

A similar scenario could explain the lack of CRISPR-Cas loci in *Streptococcus pneumoniae*, a causative agent of pneumonia. Pathogenic pneumococci are covered by a polysaccharide capsule that allows the bacterium to escape the immune system. The importance of the capsule for virulence led Griffith to the discovery of natural transformation [36] and allowed Avery to determine that DNA is the molecule that carries the genetic information of the cell [37]. In both cases, experiments relied on the acquisition of the capsule synthesis genes from heat-killed encapsulated cells by non-encapsulated pneumococci. In Griffith’s experiment, while mice injected with either heat-killed encapsulated cells or live non-encapsulated cells survive infection, co-injection leads to infection due to transformation and expression of capsule synthesis genes by non-encapsulated pneumococci. Avery’s experiment measures this transformation *in vitro* by using antiserum raised against non-encapsulated cells that prevents their planktonic growth but allows the growth of the encapsulated transformants. Capsular transformation, also known as capsule switching, is very common in nature and is the source of the more than 90 different pneumococcal serotypes. The importance of the capsule in pathogenesis has been exploited for vaccine development. Current pneumococcal

vaccines contain several different types of capsular polysaccharides and therefore *S. pneumoniae* undergoes a rapid evolution in response to the selective pressure posed by the introduction of the vaccines [38].

None of the 26 pneumococcal strains with completed genomes contain CRISPR sequences [29]. To understand the impact that CRISPR immunity could have in capsule switching, a recent study introduced the type II CRISPR-Cas system of *Streptococcus pyogenes* into *S. pneumoniae* R6, a non-encapsulated strain, and engineered an anti-capsule gene spacer [39]. By performing the Griffith and Avery experiments with this strain, investigators were able to demonstrate that the attack of transforming DNA by the CRISPR-Cas machinery could prevent pneumococcal capsule-switching, both *in vitro* and *in vivo*. Interestingly, "escaper" strains arise during mice infections that were able to acquire a capsule and cause disease. Similar to the case of antibiotic-resistant staphylococci CRISPR "escapers" (see above), these cells contained mutations in the *cas* genes that abrogated CRISPR function. Because of the artificial set up of these experiments, it is difficult to evaluate the effect of CRISPR loci in the evolution of pneumococci from their results. Nevertheless, considering the importance of natural transformation and capsule-switching for the emergence of new virulent strains of *S. pneumoniae*, it is tempting to speculate that the potential to acquire spacers targeting capsule synthesis or other virulence genes could have contributed to the selection against CRISPR-Cas loci in pneumococci.

Enterococci

Enterococcus faecalis and *Enterococcus faecium*, while natural inhabitants of the human digestive tract, are among the most common causes of antibiotic resistant hospital-acquired infections [40,41]. These organisms exhibit a propensity to incorporate mobile genetic elements such as independently replicating plasmids, prophages, and pathogenicity islands, all of which can constitute up to 25% of the genome [42]. These mobile genetic elements are rife with antibiotic resistance genes and virulence factors [43,44]. Noting the preponderance of horizontally acquired elements in these organisms and the function of CRISPR-Cas system of targeting these elements, studies explored the co-distribution of CRISPR loci and the presence of antibiotic resistance genes in *E. faecalis* and *E. faecium* strains [42,45]. While many strains harbor Type II CRISPR systems, there is a correlation between the absence of CRISPR loci and the presence of drug resistance genes. Many of the strains containing CRISPR loci were isolated prior to the widespread use of antibiotics, supporting the notion that CRISPR-Cas loss of function can occur in response to antibiotic use [42].

Group A Streptococci

S. pyogenes, or Group A Streptococci (GAS), is a major human pathogen that causes a variety of infections including pharyngitis, sepsis, and necrotizing fasciitis [46]. *S. pyogenes* strains are unique in that they have acquired a plethora of toxins and virulence factors by means of multiple prophage integrations [47]. The prophage content of *S. pyogenes* is believed to be a major determinant of the type of disease caused by specific strains and that the exchange and acquisition of prophages over time results in periodic shifts of disease manifestation and increased virulence [48]. Group A streptococci usually carry two CRISPR-Cas loci in their genomes, one type II-B, which has been shown to be functional [15], and another type I-C. A bioinformatics analysis examining the 13 available GAS genome sequences revealed that eight contained at least one CRISPR-Cas systems [49]. Interestingly, the strains that lack CRISPR sequences have the highest number of prophages (Table 2). More importantly, analysis of the CRISPR targets shows a mutually exclusive relationship between CRISPR spacer sequences and their prophage targets (Table 2 and

[49]). This suggests that CRISPR function antagonizes prophage insertion and therefore influences the evolution as well as the type of disease caused by this pathogen.

Pseudomonas

Pseudomonas aeruginosa is an opportunistic human pathogen that colonizes immune-compromised patients such as those with cystic fibrosis, cancer, or AIDS. Their pathology relies upon the formation of biofilms, surface-attached bacterial communities that can become more resistant to antibiotics than their planktonic counterparts by up to three orders of magnitude [50]. A recent study showed that about 37% of 122 *P. aeruginosa* clinical isolates harbored Type I CRISPR-Cas systems [51], that are functional against many clinical and environmental bacteriophage isolates [52]. The temperate phage DMS3, however, is able to lysogenize its *Pseudomonas* host despite the presence of a functional CRISPR-Cas system that targets it [53]. It was later demonstrated that DMS3 escape from CRISPR immunity occurs as a result of imperfect complementarity between the CRISPR spacer and the targeted region on the phage genome [52]. This phage is important because its lysogenization inhibits biofilm formation and swarming motility the *P. aeruginosa* PA14 clinical isolate. Strikingly, this inhibition requires an intact CRISPR-Cas system. While the mechanism underlying this CRISPR-Cas mediated biofilm elimination is still being investigated, the available data indicates that the biofilm defect relies upon an imperfect match between a CRISPR spacer and the *DMS3-42* gene in the phage genome [53,54]. The DMS3-42 protein is not required for inhibition of biofilms formation, suggesting that the interaction between the CRISPR-Cas machinery and the *DMS3-42* target affects the expression of the prophage genes that mediate the inhibition. This study highlights the potential for CRISPR-Cas systems to modulate bacterial physiology in unexpected ways that might mitigate their pathogenesis.

Francisella

Francisella tularensis, the causative agent of tularemia, is an intracellular pathogen known for its lethality to humans and potential for use as a biological weapon [55]. The less-virulent subspecies *novicida* is thought to be an opportunistic pathogen, and has been found to harbor a Type II CRISPR-Cas system [56]. This CRISPR-Cas system has been shown to directly enhance *F. novicida* virulence by chromosomal gene regulation and influence the outcome of a murine model of infection [57,58]. Critical for innate immunity against *F. novicida* are eukaryotic pattern-recognition receptors, which recognize bacterial lipoproteins and initiate a pro-inflammatory host response. The Type II CRISPR-Cas system in *F. novicida* was shown to use a unique small CRISPR-Cas associated RNA (scaRNA), distinct from the tracrRNA and crRNA, to repress an endogenous transcript encoding a bacterial lipoprotein. While the tracrRNA harbors the sequence complementarity to the lipoprotein transcript that mediates the down-regulation of this gene, the scaRNA has homology to tracrRNA sequences and is required for repression. These RNA-RNA interactions, mediated by Cas9, result in a reduction of the lipoprotein transcript, leading to less expression of this antigen and a decrease in the host pro-inflammatory response. *F. novicida* strains harboring mutations in the CRISPR-Cas system or the scaRNA were attenuated during murine infection, and mice vaccinated with these attenuated strains were protected from future infection. This seminal study establishes a direct role for CRISPR-Cas in regulating the interaction between the bacteria with its eukaryotic host.

Conclusions

The specific cases described in this review reveal a love-hate relationship between bacterial pathogens and their CRISPR-Cas systems. On the one hand, the ability of CRISPR

immunity to prevent the transfer of antibiotic resistance and virulence genes into pathogens can reduce the evolvability of the pathogen. On the other, CRISPR-Cas systems can be repurposed to regulate gene expression and enhance pathogenesis. The application of novel sequencing technologies to epidemiological studies will allow us to understand the full effect of CRISPR-Cas loci on the evolution of important human pathogens such as *S. pneumoniae*, *S. aureus* and *E. fecaelis*. The role of CRISPR-Cas systems in the modulation of virulence is certainly a new and exciting area of research. In addition to the case of *F. novicida*, a number of recent observations support the influence of CRISPR immunity on host-pathogen interactions, although the mechanisms remain obscure. For example, deletion of *cas9* from the Type II CRISPR-Cas system in *Nisseria meningitidis* resulted in its reduced ability to adhere to, invade, and replicate within human epithelial cells, leading to a defect in survival of the pathogen [57]. Similarly, Cas9 was shown to be critical for host interactions in *Campylobacter jejuni* [59]. These effects are most likely mediated by a scaRNA, however the gene(s) down-regulated are unknown. Also Cas2, a component of the Type II CRISPR-Cas adaptation machinery in *Legionella pneumophilia*, was shown to facilitate infection of its amoeba host, a parasitic interaction that is required for *L. pneumophilia* proliferation in aqueous environments and transmission to humans [60]. Taken together, these examples demonstrate the remarkable versatility of CRISPR-Cas systems and warrant future investigations focused on the roles of CRISPR-Cas that extend beyond the canonical interference against foreign nucleic acids.

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Highlights

- CRISPR-Cas systems protect bacteria from bacteriophages and other mobile genetic elements.
- CRISPR-Cas systems can be barriers to horizontal gene transfer, reducing the evolvability of pathogens.
- CRISPR-Cas systems can increase virulence by modulating gene expression.
- These different functions have opposite effects on the survival of different pathogens.
- As a result, CRISPR-Cas systems are not universal in bacterial pathogens.

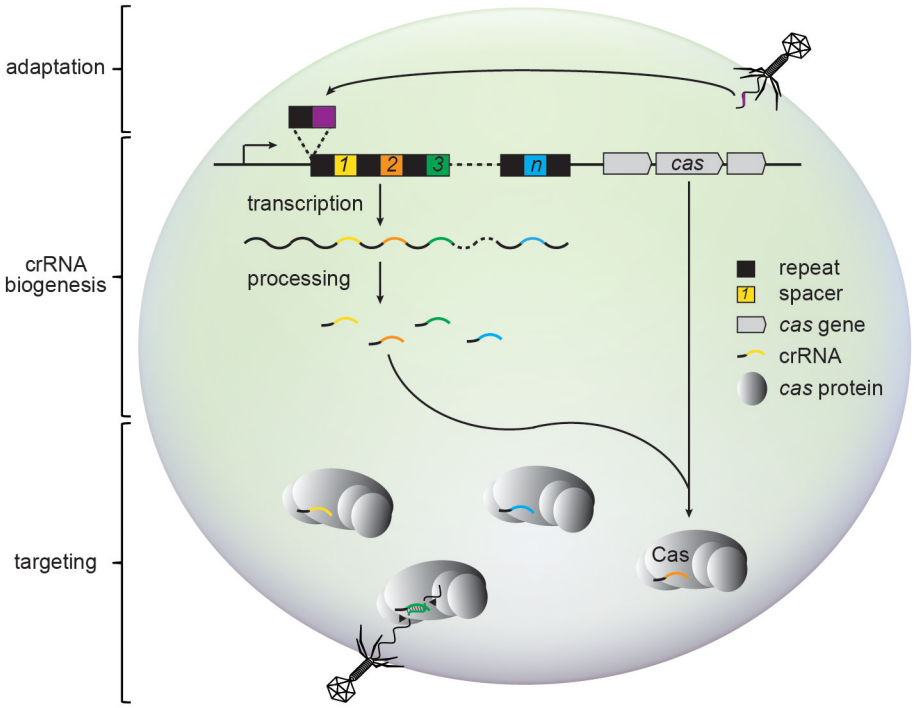

Figure 1. The three stages of CRISPR-Cas immunity

During adaptation, foreign nucleic acids stimulate prokaryotes to insert invader-derived sequences called spacers (colored, numbered squares) in between DNA repeats (black squares) found in the CRISPR locus. During crRNA biogenesis, these repeats and spacers are transcribed into one contiguous precursor crRNA, which is processed to liberate small interfering crRNAs containing a single spacer sequence. During targeting, these mature crRNAs assemble with *cas* gene products and direct the destruction of matching invasive nucleic acids. CRISPR immunity against both phage and plasmid DNA, as well as RNA have been observed.

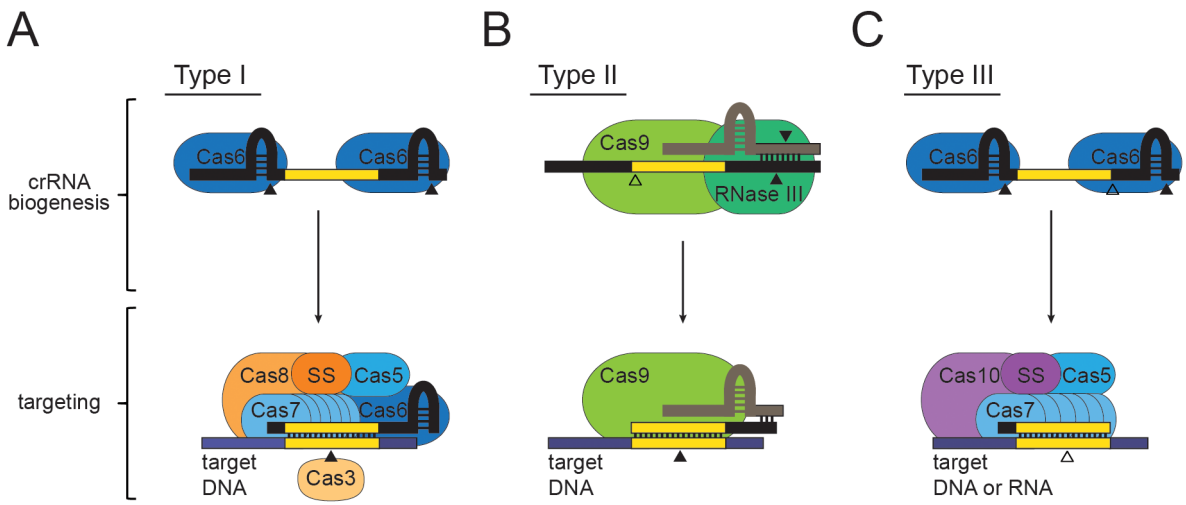

Figure 2. The Cas protein families required for crRNA biogenesis and targeting in the three CRISPR-Cas types

(**A**) In Type I CRISPR-Cas systems, a Cas6 endoribonuclease cleaves within repeat sequences (colored black) to generate mature crRNAs. CrRNAs then assemble with a targeting complex that includes Cas6, the Type I signature subunit Cas8, and members from the Cas5 and Cas7 families. An independent small subunit (SS) may be present in some subtypes, or found fused to Cas8. The DNA target (colored blue) is cleaved by Cas3, a protein that is not associated with the complex. (**B**) In Type II CRISPR-Cas systems, a trans-encoded CRISPR-RNA (tracrRNA, colored beige) binds crRNA repeats through base-pair complementarity, and facilitates RNase III-mediated cleavage of both RNAs. CrRNA biogenesis requires additional cleavage by an unknown nuclease to generate mature crRNAs. A single large multi-domain protein, Cas9, is required for both crRNA biogenesis and DNA target cleavage. (**C**) In Type III CRISPR-Cas systems, crRNA biogenesis is accomplished by Cas6-mediated cleavage within repeats and additional trimming by an unknown nuclease. The Type III targeting complex contains Cas10, the large signature subunit, Cas5, and distinct members of the Cas7 family. An additional subtype-specific small subunit (SS) is also a present in Type III complexes. Targeting against DNA and RNA in Type III systems is catalyzed by an unknown nuclease. Solid arrows represent nucleolytic cleavage events carried out by the protein on top of which they appear, and open arrows indicate cleavage events carried out by unknown nucleases.

Table 1

CRISPR loci and their subtypes in common bacterial pathogens

Species	Disease	CRISPR[a]	Subtype[b]
Bacillus anthracis	Cutaneous, pulmonary or gastrointestinal anthrax	0/6	
Bordetella pertussis	Whooping cough	0/3	
Borrelia burgdorferi	Lyme disease	0/4	
Brucella abortus, B. canis, B. melitensis, B. suis	Brucellosis	0/14	
Campylobacter jejuni	Acute enteritis	10/11 (ref)	II-C
Chlamydia trachomatis	Nongonococcal urethritis, lymphogranuloma venereum, trachoma	0/21	
Chlamydophila psittaci, C. pneumoniae	Psittacosis	0/11	
Clostridium botulinum	Botulism	14/14	III-B
Clostridium difficile	Pseudomembranous colitis	9/9	I-B
Clostridium perfringens	Gas gangrene, acute food poisoning	0/3	
Clostridium tetani	Tetanus	1/1	I-A/B[c]
Corynebacterium diphtheriae	Diphtheria	13/13	II-C, I-E
Enterococcus faecalis	Nosocomial infections	4/5	II-A
***Escherichia coli* (enterohemorrhagic (EHEC))**	diarrhea, hemorrhagic colitis, hemolytic-uremic syndrome	14/15	I-E
Francisella tularensis	Tularemia	1/14	II-B
Gardnerella vaginalis	Bacterial vaginosis	2/3	I-E
Haemophilus influenzae	Bacterial meningitis, upper respiratory tract infections, pneumonia, bronchitis	0/9	
Helicobacter pylori	Peptic ulcer, risk factor for gastric carcinoma	17/49	-[c]
Legionella pneumophila	Legionnaire's Disease	3/10 (ref)	II-B
Leptospira interrogans	Leptospirosis	2/3	-[c]
Listeria monocytogenes	Listeriosis	19/27	I-B, II-A
Mycobacterium leprae	Leprosy (Hansen's disease)	0/2	
Mycobacterium tuberculosis	Tuberculosis	19/19	III-A
Mycoplasma pneumoniae	Mycoplasma pneumonia	0/3	
Neisseria gonorrhoeae	Gonorrhea	1/3	I-C
Neisseria meningitidis	Meningococcal disease including meningitis	12/14	II-C
Pseudomonas aeruginosa	Pseudomonas infection (localized to eye, ear, skin, urinary, respiratory or gastrointestinal tract or CNS, or systemic with bacteremia)	5/8	I-F
Rickettsia rickettsii	Rocky mountain spotted fever	0/8	
Salmonella typhi	Typhoid fever type salmonellosis (dysentery, colitis)	3/4	I-E
Salmonella typhimurium	Salmonellosis with gastroenteritis and enterocolitis	8/9	I-E
Shigella sonnei	Bacillary dysentery, Shigellosis	2/3	I-E
Staphylococcus aureus	Coagulase-positive staphylococcal infections (skin infections, acute infective endocarditis, septicemia, necrotizing pneumonia, toxic shock syndrome)	2/39	III-A

Species	Disease	CRISPR[a]	Subtype[b]
Staphylococcus epidermidis	Infections of implanted prostheses, e.g. heart valves and catheters	1/2 (ref)	III-A
Staphylococcus saprophyticus	Cystitis in women	0/1	
Streptococcus agalactiae	Meningitis and septicemia in neonates, endometritis in postpartum women	4/9 (ref)	II-A, I-C
Streptococcus pneumoniae	Acute bacterial pneumonia and meningitis in adults, otitis media and sinusitis in children	0/20	
Streptococcus pyogenes	Streptococcal pharyngitis, scarlet fever, rheumatic fever, impetigo, necrotizing fasciitis	11/18 (ref)	II-A, I-C
Treponema pallidum	Syphilis	0/8	
Vibrio cholerae	Cholera	1/7	I-E
Yersinia pestis	Bubonic and pneumonic plague	12/12	I-F
Yersinia pseudotuberculosis	Gastroenteritis	4/4	I-F

[a] The number of CRISPR-containing strains of the total number of strains with complete genomes is shown as reported by CRISPRdb (http://crispr.u-psud.fr/crispr/). A CRISPR-containing strain is defined as one with a CRISPR locus that harbors at least two spacers. Numbers were corroborated and corrected where necessary using the distribution of CRISPR-Cas loci reported by Makarova et al. (ref).

[b] The most predominant CRISPR subtype among strains is noted first, followed by the less prevalent. Subtypes were confirmed by indicated references.

[c] A definitive determination cannot be made based on the available information.

Table 2

CRISPR and prophage content of GAS genomes.

Strain[a]	Serotype	CRISPR[b]	Prophages in the genome[c]	Prophages targeted by CRISPR[d]		Ref.
SF370	M1	9	370.1 – 4	10270.1, 2 315.2, 3, 4, 5 SPsP2, 3, 4, 5 10750.1, 2, 3 10394.3, 4, 5	9429.2 8232.2, 3, 5 6180.2 NZ131.3	[61]
MGAS9429	M12	9	9429.1 – 3	5005.1, 2 315.1, 2 SPsP5, 6 Man.1, 4	8232.1, 2, 5 6180.1 NZ131.3	[62]
NZ131	M49	9	NZ131.1 - 3	370.1, 3 5005.2 10270.3 315.3, 4 SPsP3, 4 10750.3	Man.1, 2 10394.4, 5, 6 2096.1 8232.2, 4, 5 6180.2	[63]
MGAS2096	M12	8	2096.1 – 2	5005.1 315.1, 2 SPsP5 Man.1, 4	8232.1, 2, 5 6180.1 NZ131.3	[62]
MGAS5005	M1	7	5005.1 – 3	370.1 10270.1, 2, 3 315.2, 4, 5 SPsP2, 4, 5 10750.1, 2	Man.1, 3, 4 10394.6 9429.1 8232.2, 5 NZ131.3	[64]
MGAS10270	M2	6	10270.1 – 5	370.1 315.1, 2, 3 SPsP3, 4, 5 10750.3	Man.2, 3, 4 10394.3, 5 8232.1, 2, 3 6180.1	[62]
MGAS6180	M28	5	6180.1 – 4	370.2, 3 5005.2 315.3, 5 SPsP2, 4, 5	10750.3 10394.4, 5 9424.2 8232.3, 4, 5	[65]
MGAS10750	M4	5	10750.1 – 4	370.3 315.5 SPsP2	Man.1 8232.4	[62]
MGAS315	M3	-	315.1 – 6	-		[66]
SSI-1	M3	-	SPsP1 – 6	-		[67]
Manfredo	M5	-	Man.1 – 5	-		[68]
MGAS10394	M6	-	10394-1 – 8	-		[69]

Strain[(a)]	Serotype	CRISPR[(b)]	Prophages in the genome[(c)]	Prophages targeted by CRISPR[(d)]	Ref.
MGAS8232	M18	-	8232.1 – 5	-	[70]

(a) Strains are ordered from highest to lowest total number of CRISPR spacers.

(b) Number of spacers is indicated.

(c) Prophages and prophage remnants are indicated.

(d) Prophages carry the name of the host strain followed by a serial number. Note that there is a mutually exclusive relationship between CRISPR-Cas systems and prophage content. For example, the CRISPR spacers present in strain SF370 match prophages present in other strains, but none of the 370.1-4 endogenous prophages.